APPENDIX
IN THE SPECIFICATION

The immobilization antigen of *I. Multifiliis* (i-AgI) consisted of three tandem repeats in its central region. Three DNA segments of similar length were designed to cover a complete repeat I of i-AgI. For each segment, two oligonucleotides, representing both coding and noncoding strands, were synthesized. To ensure efficient intermolecular annealing, the two oligonucleotides contained 12 complementary nucleotides at their 3' ends (Fig. 1B). Restriction sites were engineered at the 5' ends of each oligonucleotide to facilitate cloning. To generate double-stranded DNA from the oligonucleotides, 10 :l each of the corresponding two oligonucleotides were mixed, at the same concentration (1 : g/ : l), with 5 : l of 10 X Klenow buffer (500mM Tris, pH7.2, 100mM $MgSO_4$, 1mM dithiothreitol) and 18 : l distilled water. The reaction mixture was heated and maintained at 80°C for 5 min and then allowed to cool to 37°C, followed by addition of 5 :l of 2.5mM deoxyribonucleotides (dNTP) and 2 :l of Klenow fragment (Promega) to initiate DNA synthesis at room temperature. After 40 minutes EDTA was added to a final concentration of 10 mM to stop the reaction,and this was followed by incubation at 75°C for 5 min to inactivate the Klenow enzyme. The double-stranded DNA segments were purified by phenol/chloroform extraction and ethanol precipitation, and dissloved in 50 :l of distilled water. To assemble the three segments into a longer DNA fragment, half of double-stranded segments 1, 2 and 3 were double digested with BamHI-KpnI, KpnI-Sau3A1 and Sau3A1-EcoRI, respectively. The digested segments were recovered by gel purification, using low-melting-temperature agarose, and dissloved in 25 :l of water. About 0.1 :g of digested segment 1 was ligated into 0.1 :g of pBluescript vector (stratagene) that had been predigested with BamHI and KpnI. The ligation was performed overnight at 4°C in a 20 :l reaction mixture. Similarly, 0.2 :g each of segment 2 and segment 3 were ligated together into pBluescript vector

(Stratagene) that had been predigested with KpnI and EcoRI. 10 :l of each ligation reaction mixture was transferred into 100 :l of *E. coli* competent cell (XL1-Blue strain, Startagene) according to the standard protocol described by Sambrook, J., Fritsch E.F. and Maniatis,T. ("MOLECULAR CLONING, A LABORATORY MANUAL" 2nd edition, Cold Spring Harbor Laboratory Press, New York, 1989). The resulted plasmids were isolated with Qiagen Plasmid Isolation Kit (Qiagen) and sequenced from both ends for confirmation. The verified segment 1 and segments 2 and 3 were removed from pBluescript by digestion with BamHI-KpnI and KpnI-EcoRI respectively. 0.1 :g of verified segment 1 and segments 2 and 3 each were ligated into 0.1 :g of pGEX2T (Pharmacia) vector cut with BamHI and EcoRI. The volume of 10 :l ligation reaction was transformed into *E. coli* (XL1-Blue strain, Stratagene), and the resulting construct was called pGST-iAgI. Its sequence was confirmed again by sequencing with the primer 5'-TAGCATGGCCTTTGCAG-3', upstream [SEQ. ID No. 19] of the cloning site of pGEX2T vector.